

# **Fluid Mechanical and Chemical Cues in Thin Layers: Role in Organizing Zooplankton Aggregations**

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## **LONG-TERM GOALS**

We are examining the physical and chemical conditions that are conducive to the formation of zooplankton aggregations to help address the prevalence and importance of thin layers in the world's oceans. The goal of the laboratory experiments is to define the mechanisms by which zooplankton are attracted to thin layers, and to determine the properties of thin layers that evoke aggregative responses in copepods.

## **OBJECTIVES**

A series of experimental treatments are used to isolate the physical and chemical signals that induce zooplankton orientation. The objectives include: 1) improving our understanding of formation and persistence of thin layers and zooplankton aggregations; 2) identifying the balance between physical forcing and biological responses in thin layer formation; 3) generating data on zooplankton responses that are required for individual-based models of aggregation to thin layer signals; 4) providing information to field studies about the range of physical measurements that must be performed in order to characterize thin layers and to evaluate their spatial and temporal persistence; and 5) helping to identify target field sites by determining the thresholds at which relevant signals induce aggregations.

## **APPROACH**

The hypothesis to be tested is that fluid mechanical and chemical signals characterizing thin layers actively modify the behavior of zooplankton to attract them to these regions and result in significant accumulation of zooplankton near, or in, these features. A series of experimental treatments isolate the chemical and hydrodynamic signals that induce zooplankton orientation. Experiment 1 subjects copepods to isolated layers of velocity gradient, density gradient, chemical exudate, or biological (phytoplankton cells) that bracket natural conditions in thin layers. This experiment allows us to identify which components of thin layers are responsible for aggregative behavior. Experiment 2

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assays copepod responses to combined velocity and chemical layers, density and velocity layers, density and chemical layers, and velocity, density, and chemical layers because these combinations more accurately model *in situ* conditions. These experiments allowed us to identify important interactive effects of the gradients on copepod behavior.

Yen (PI) is responsible for overall coordination of fluid mechanical, behavioral and sensory experiments. Webster (co-PI) is responsible for the design and implementation of flow and odor field analysis and kinematic analysis of animal trajectories. Weissburg (co-PI) is responsible for statistical analysis of behavior data and ecological interpretation of the data. Brock Woodson (graduate research assistant) has been performing the physical measurements and animal observations. Katie Rasberry (graduate research assistant), Jen Sehn, Genevra Pittman, and Jenny Huang (undergraduate research assistants) have been performing animal observations. Genevra Pittman and Jenny Huang were supported by the NSF REU site grant to Georgia Tech.

## WORK COMPLETED

Model thin layers with isolated gradients of velocity, density, food, and chemical exudates from sympatric phytoplankton species as well as all combinations of velocity, density, and chemicals that mimic field conditions (Dekshenieks et al. 2001, Cowles 2004) have been established in our specially-designed flume apparatus at Georgia Tech (Fig. 1). Sample profiles for the combined velocity-density-concentration layer are shown in Fig. 2. The velocity field is measured with particle image velocimetry (PIV) and the density and concentration fields are measured via the laser-induced fluorescence (LIF) technique (Woodson et al. 2005, 2006). Both laser-based measurement techniques are non-intrusive and hence do not disturb the layer during data collection.

Copepods are collected around the U.S.A. and shipped overnight to Atlanta where they are sorted by species and transferred to holding tanks for a minimum of two days before experiments. All experiments are performed in an environmental room that is adjusted to ambient water temperature based on location and season of the region where the animals were collected (typically 12-15°C). Adult copepods of several species have been evaluated in the past 3 years: *Acartia tonsa*, *Temora longicornis*, *Candacia ethiopica*, *Labidocera madurae*, *Calanus finmarchicus*, *Calanus pacificus*, *Neocalanus plumchrus*, *Eurytemora affinis*, and *Metridia pacifica*. *Acartia*, a predominantly mechanoreceptive copepod, is compared to *Temora*, a chemoreceptive copepod, thus comparing sensory modalities. *Candacia* and *Acartia* are carnivorous, or omnivorous, while the others are more herbivorous, thus comparing trophic status. *Calanus finmarchicus*, the dominant N. Atlantic species, is compared to *C. pacificus*, the Pacific species, to assess intra-genera differences. *Neocalanus* serves as a cold-water species (5°C) and *Eurytemora* serves as a euryhaline estuarine species.

Species-specific groups of fifty copepods are added to the tank and aggregated at the surface or bottom using a fiber-optic light source until the start of experiments. Once the animals have adjusted to the tank (approximately two hours) the experiment is begun. Animal positions in a 10-cm × 10-cm observation section are recorded on video tape (VHS) for a period of two hours. Individual paths of swimming organisms are converted to data files using ExpertVision software. Analysis of individual paths consists of calculating residence time in the layer, swimming speeds, and turn frequency. For the velocity gradient layer data, the swim speed was defined as the speed relative to the flowing fluid. Turn frequency is the number of turn events per second where a turn event is defined as a change in trajectory of more than 15 degrees. Each of these factors is computed for overall path, pre-contact, and post-contact with the layer. Statistical comparisons of proportional residence time are done using

either a multi-way nested ANOVA or single-factor ANOVA combined with post-hoc tests. Swimming speeds and turn frequency are compared for differences between pre- and post-contact using a repeated measures ANOVA design. Statistical comparisons between controls and treatments are not conducted between swimming speed and turn frequency because sex ratios were not controlled between experiments and can thus impact these factors.

## RESULTS

Most tested species of copepods exhibited behaviors congruent with excited area-restricted search behavior in response to the velocity gradient layer (Figs. 3 and 4, Woodson et al. 2005). Significant increases in proportional residence time in response to the velocity gradient layer resulted from increased swimming speeds and turn frequency suggesting that these animals were positively excited by the presence of the velocity gradient. Species specific threshold shear levels of  $0.035\text{--}0.06\text{ s}^{-1}$  for *T. longicornis* and  $0.03\text{--}0.06\text{ s}^{-1}$  for *A. tonsa* constitute conditions where we observe changes in zooplankton behavior and are well below the thresholds currently known to elicit escape responses (Fields and Yen 1997). Establishing threshold shear strain rates in the laboratory is critical to our understanding of conditions that elicit zooplankton aggregations because the velocity gradients are quantified at a level of accuracy that is not achievable in the field. The exquisite sensitivity of zooplankters suggests that aggregations to velocity gradients may occur under a wider range of conditions than is currently known based on field studies.

Velocity gradients appear to act as an initial cue for aggregation, thus instigating an excited area-restricted search. Copepods cease local searching and continue ‘random-directed’ movement over time, if no further cue is present (i.e. food or chemical). *Eurytemora affinis* is currently the lone exception; this species does not display a significant response to the velocity gradient layer, suggesting that species specific responses, as well as physical conditions, are responsible for the composition and characteristics of zooplankton aggregations to thin layers (Fig. 4).

*T. longicornis* trajectories for the velocity gradient layer treatment indicate that these copepods have a preferred orientation of their body axis relative to the flow (Fig. 5). Our current imaging capability does not facilitate extracting the body axis orientation, but in the case of the cruise swimmer, *T. longicornis*, the body axis is fairly well correlated with the swim direction (not true for the hop-sink traveler, *A. tonsa*). The data show that prior to contact with the layer, the distribution of orientation angle is nearly uniform (i.e. no preference). After contact, copepod orientation is preferentially aligned near  $0^\circ$  (Fig. 5). Thus, the body axis of *T. longicornis* is preferential aligned with the flow direction in the thin layer mimic and the antennules (which carry the mechanoreceptive setae) are preferentially aligned with the velocity gradient direction. The specific advantage of this alignment is not known, but preferential alignment of copepods may affect acoustic backscatter measurements potentially useful for quantifying zooplankton aggregations.

Responses to the density gradient layer were strikingly different. Individuals rarely cross the density gradient layer, which suggests density changes may act as barriers to vertical migration for copepods, thus resulting in aggregations at these boundaries due to a physical restriction rather than attraction (Lance 1962; Harder 1968; Woodson et al. 2005). The specific response is species dependent, however. *Temora longicornis* and *E. affinis*, for instance, show increased residence time in the layer, whereas other species, such as *A. tonsa*, did not increase residence time (Fig. 6). Figure 7 demonstrates that the number of *A. tonsa* individuals crossing the density gradient layer is non-linearly dependent on the strength of the density jump. Sigmoidal regression was used in order to estimate the

lower response threshold. Regression of the number of individuals crossing versus density jump strength revealed a lower threshold boundary of  $\Delta\sigma_t = 0.8$  (i.e. the curve begins to decrease near this value). The response to the density gradient approaches approximately 75% of the population not crossing the gradient by  $\Delta\sigma_t = 2.0$ .

Responses to chemical layers are similar to, but stronger than, those observed for the velocity gradient layers (Fig. 8). *Temora longicornis* and *Acartia tonsa* increased swimming speed and turn frequency in response to chemical exudates from phytoplankton resulting in an increase in proportional residence time (Woodson et al. 2006). *A. tonsa* significantly increased proportional residence time in response to chemical exudate concentration ( $df = 449$ ,  $F = 20.87$ ,  $p < 0.001$ ; single-factor ANOVA) with significant differences from the control value occurring at  $158 \mu\text{g C L}^{-1}$  (difference = 0.174;  $MSD_{0.05,449,1} = 0.098$ ;  $p < 0.001$ ). This suggests a behavioral threshold near  $150 \mu\text{g C L}^{-1}$  for *A. tonsa* responses to *Tetraselmis* sp. exudates. This concentration is above background levels and below peak concentrations reported for *in situ* thin layers and patches (Cowles 2004), which suggests that *T. longicornis* can chemically sense phytoplankton assemblages. Interactions between cues in combined layers revealed that the response to chemical exudates was stronger than responses to physical gradients.

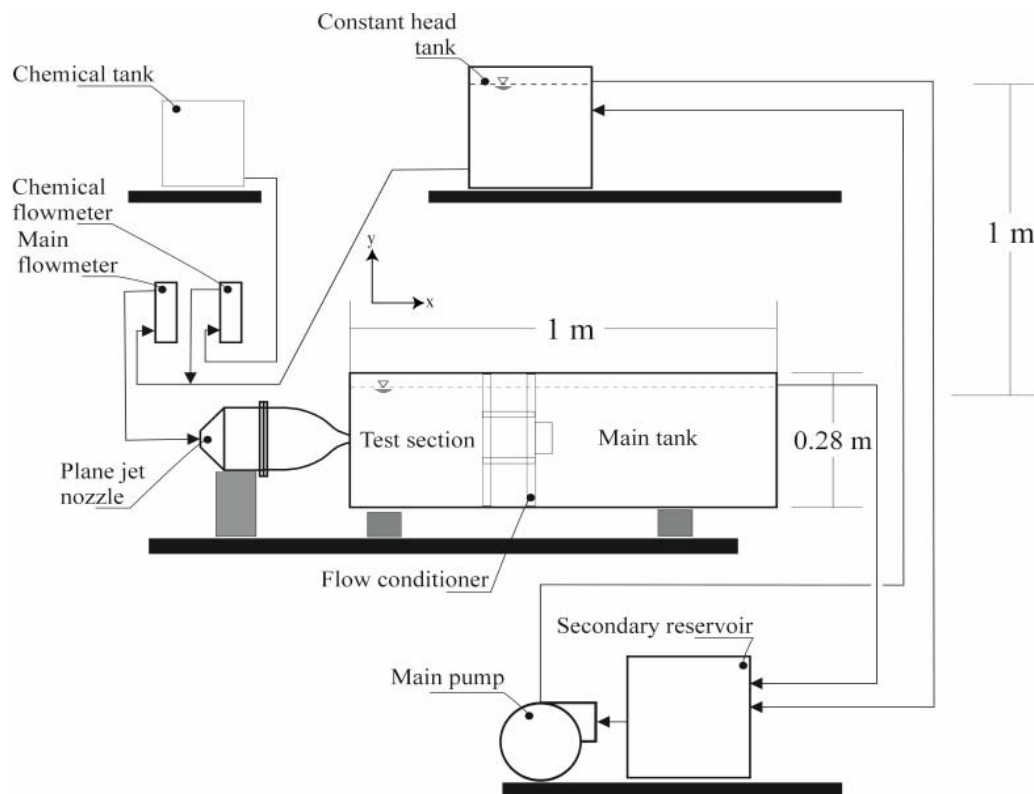
In response to food layers, copepods increased proportional residence time in the layer (Fig. 10), but the mechanism differed from the excited area-restricted search behavior demonstrated in the velocity and chemical layers. In the food layer treatment, copepods initially began search behavior, but quickly began feeding, suggesting a secondary cue (mechanical or chemical) provided by the presence of phytoplankton cells. Their swimming speed and turn frequency decreased as a result of the feeding behavior.

These behaviors are congruent with our hypotheses of hierarchical responses to cues associated with thin layers. The existence of a cue hierarchy was further supported by the significant interaction between chemical exudates and the physical gradients (proportional residence time data for *A. tonsa* shown in Fig. 3). That is, chemical cues paired with either a velocity or density gradient significantly enhanced proportional residence time as compared to the isolated treatments. But, the response to the chemical cue was similar regardless of whether it was paired with a physical gradient. Further, the presence of a velocity gradient layer in a homogeneous environment of chemical exudates did not invoke an excited area-restricted search behavior. The above interactions suggest that the behavioral response to combined factors was dominated by the presence of the chemical component. For all combined treatments, density gradients continued to act as a boundary, and thus may indirectly aid the individual in remaining in a region of interest and possibly improving search efficiency. *Temora longicornis* displays a similar, but slightly distinct hierarchical responses as explained in Woodson et al. (2006). *Eurytemora affinis* also demonstrates a modified cue hierarchy with density gradient acting as the initial cue rather than velocity gradient. In this case, the response to the density gradient is not negated in the presence of a homogeneous environment of chemical exudates, which highlights another species-specific attribute.

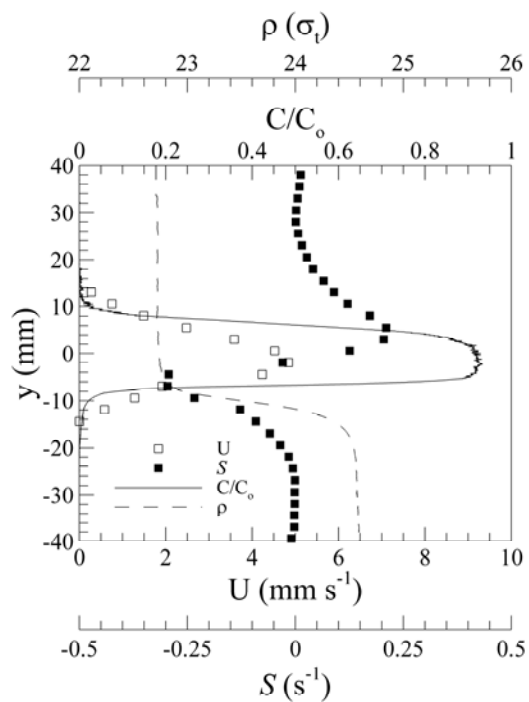
## IMPACT/APPLICATIONS

Thin layers provide a complex combination of gradients in fluid velocity and density, chemical composition, and plankton composition. Here, with our series of experimental treatments isolating chemical and hydrodynamic signals, we have documented a hierarchy in the response of copepods that evoke the aggregative responses necessary to lead to thin layer formation and persistence. Not only do copepods detect fluid velocity gradients, chemical scents from their phytoplanktonic food enhance their aggregative response. These responses appear species-specific, with some copepods showing strong active aggregative responses whereas another showed limited response to certain cues. Such variations in threshold sensitivity to thin layer cues can lead to species specific layering or, more broadly, species-specific distributions over horizontal space. The complexity of the behavioral response to the combinations and intensity of water-borne cues continue to support our efforts to use the copepod as a biosensor of small-scale biological-physical-chemical signals in the sea.

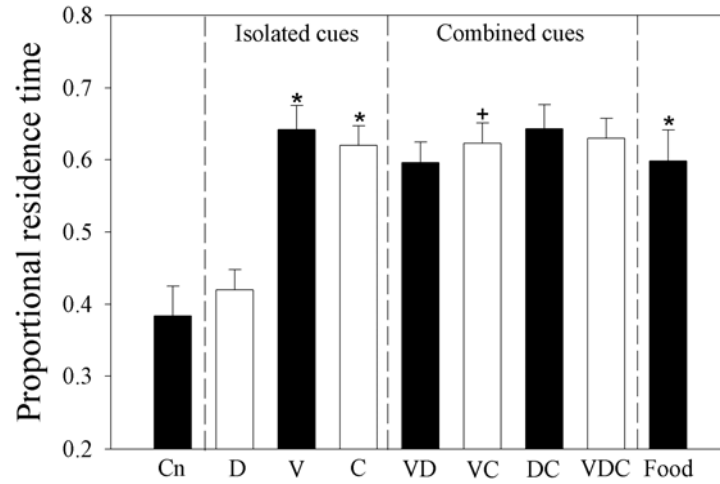
Our experimental results also indicate the importance of species composition on the balance between physical forcing and biological responses in thin layer formation. When biological responses are a significant factor, individual-based behavior models can be used in conjunction with Lagrangian transport models to understand or predict aggregation to small-scale chemical and fluid structures (Yamazaki et al. 2002). Establishing the physical conditions under which zooplankton orient to fluid velocity or chemical gradients therefore will aid modeling efforts by outlining when these models must incorporate biological responses and active movement of individuals. Further, our experiments supply data on movement velocities, turning rates and other response variables that are required for these individual-based models. Alternatively, determining when zooplankton responses are not the dominant driving force will establish a framework in which thin layers may be modeled via physical transport processes that affect organisms. Under these conditions, distributions of organisms can be modeled in a manner similar to other passive scalar quantities such as heat or chemical concentration. The relative simplicity of models that do not require explicit biological assumptions makes it critical that we establish when they can be reliably employed.



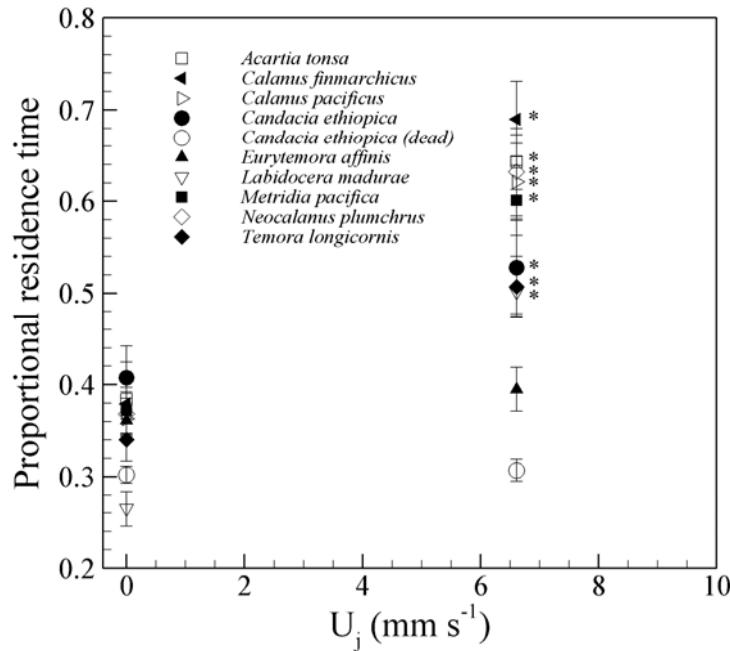
**Fig. 1. Sketch of the laboratory thin layer apparatus.**



**Fig. 2. Velocity, shear strain rate, density, and concentration profiles for the combined velocity-density-chemical layer treatment.**

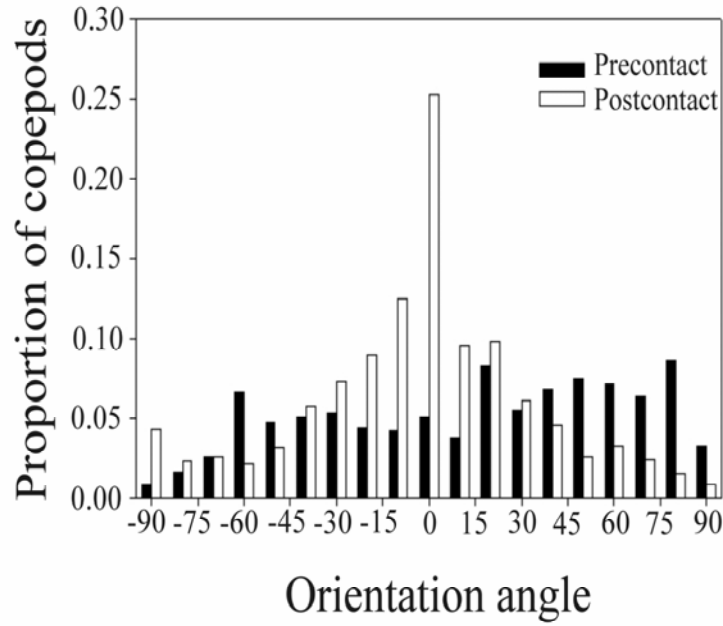


**Fig. 3. Proportional residence time in the presence of individual cue and combined cue layers for *A. tonsa*.** Layer constituents are velocity (*V*), density (*D*), and chemical exudates (*C*) and phytoplankton (*Food*). For isolated cues, \* indicates a significant difference relative to the control (*Cn*) as determined with nested ANOVA ( $p < 0.05$ ). For combined cues, + indicates significant interactive effects between layer constituents for combined cue layers.

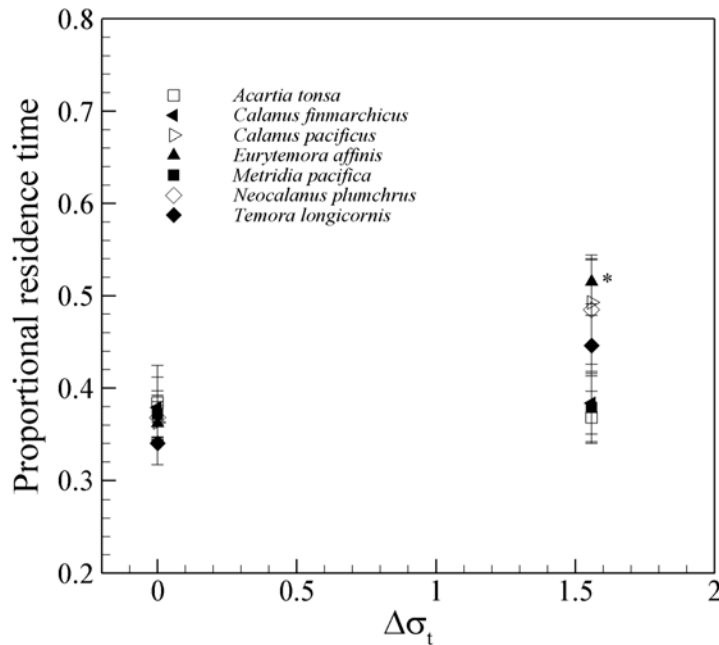


**Fig. 4. Proportional residence time of adults in the velocity gradient layer treatment ( $U_j = 6.7 \text{ mms}^{-1}$ ). Control experiment is at  $U_j = 0 \text{ mms}^{-1}$ .** \* indicates significant differences between treatment and controls ( $p < 0.05$ ). *Eurytemora affinis* and dead specimens of *Candacia ethiopica* are the only tested species that did not significantly respond to the velocity layer treatment with increased residence time.

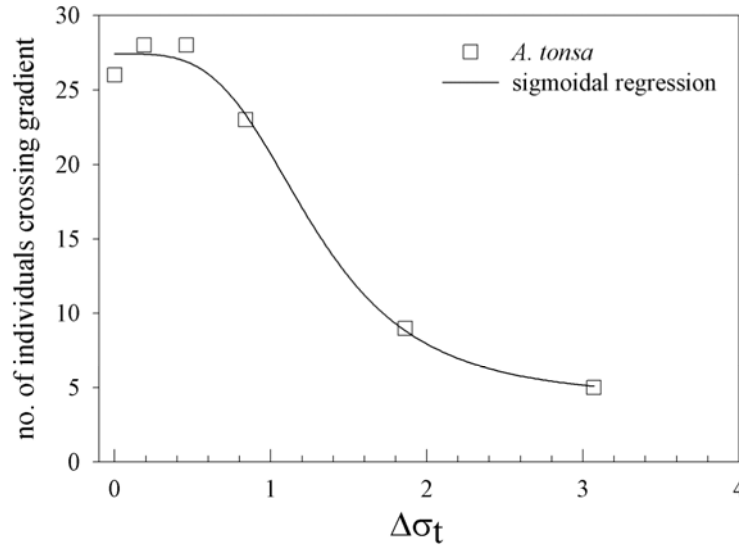




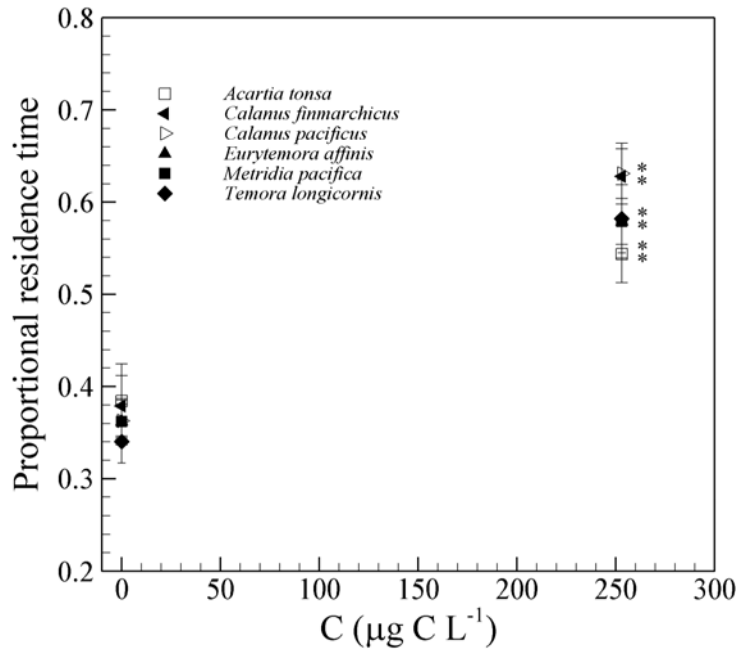
**Fig. 5.** Distribution of orientation angle for *Temora longicornis* showing marked difference before and after contact with the isolated velocity gradient layer. The orientation angle is defined such at 0° is horizontal (either upstream or downstream) and +90° is vertically upward.



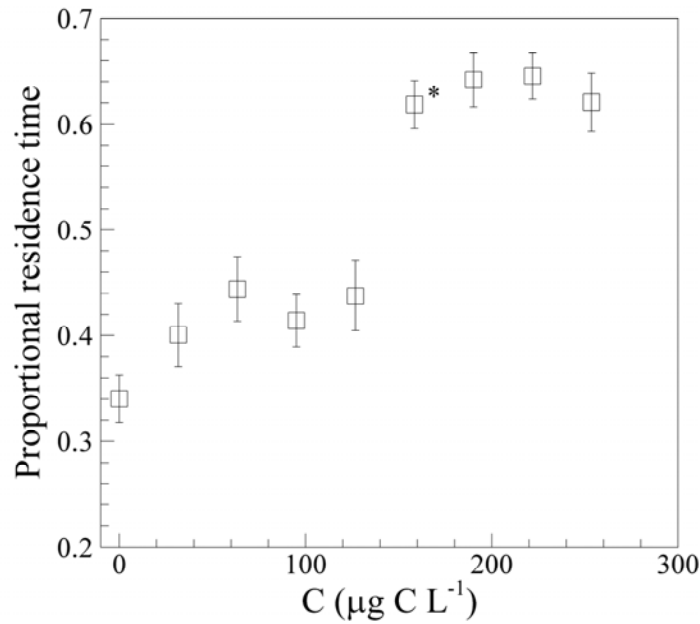
**Fig. 6.** Proportional residence time of adults in the density gradient layer treatment. Controls correspond to a constant salinity layer (32 ppt). Treatments contain a 2 ppt salinity change in the middle of the tank. \* indicates significant difference ( $p < 0.05$ ) of treatment values over control values. *Eurytemora affinis* increased residence time for the density layer treatment and changed swimming characteristics, whereas the other species did not.



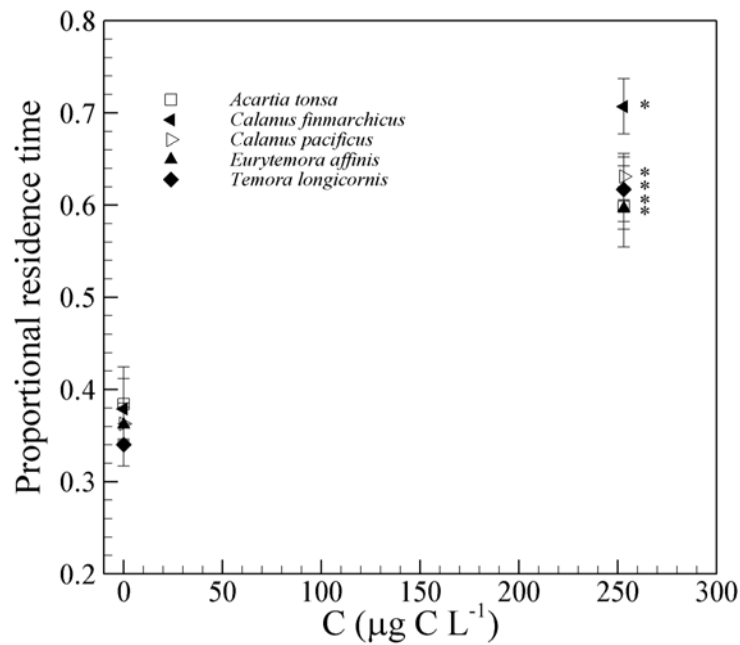
**Fig. 7. Behavioral response of *A. tonsa* to the density gradient layer treatment is represented by the number of individuals that cross the gradient layer ( $R^2 = 0.972$ ,  $p = 0.094$ ). Density gradients are a result of a change in salinity (0, 0.25, 0.5, 1, 2, 4 ppt) corresponding to a change in  $\Delta\sigma_t$  (0, 0.2, 0.4, 0.8, 1.8, 3.1, respectively). Sample sizes are the same for all gradient levels ( $n = 40$ ). Threshold level was  $\Delta\sigma_t = 0.8$  for *A. tonsa* as defined by the point where the sigmoidal curve begins to decrease.**



**Fig. 8. Proportional residence time in the phytoplankton chemical exudate (*Tetraselmis* sp.) layer treatment. Concentration is based on equivalent biomass of phytoplankton in  $\mu\text{g C L}^{-1}$ . Controls correspond to the absence of exudate ( $0 \mu\text{g C L}^{-1}$ ). \* indicates significant difference ( $p < 0.05$ ) of treatment values over control values.**



**Fig. 9.** Proportional residence time of *A. tonsa* in the presence of a chemical exudate layer of *Tetraselmis* sp. Concentration of exudate is calculated as the equivalent biomass concentration ( $\mu\text{g C L}^{-1}$ ). \* indicates significant difference ( $p < 0.05$ ) of treatment value versus preceding value via S-N-K post-hoc test.



**Fig. 10.** Proportional residence time in the food (*Tetraselmis* sp.) layer treatment. Controls correspond to the absence of food ( $0 \mu\text{g C L}^{-1}$ ). \* indicates significant difference ( $p < 0.05$ ) of treatment values over control values.

## RELATED PROJECTS

The PIs have several projects that focus on the interdependence of copepod behavior, flow, turbulence, and mechanoreception. The observations and results of these projects reinforce and supplement the current project. The currently funded projects are:

“Biologically-generated flow by plankton: Numerical simulation and experiments” PI: Yen. Sponsor: NSF Fluid Mechanics. July 2006 – June 2009

“Mechanoreception in marine copepods: coding complex fluid disturbances” PIs: Fields and Weissburg. Sponsor: NSF Integrative Biology and Neuroscience. Jan 2003 – Dec 2006.

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